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Institute for Reference Materials and Measurements

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REFERENCE MATERIALS

The method-specific certification of the cholesterol and triglyceride contents of a pure and an adulterated butter fat reference material

BCR-632A and BCR-632B

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ABSTRACT

This report describes the preparation of a set of two butter fat reference materials (pure and adulterated butter fat) and the measurement exercises that led to the method-specific certification of the content (relative mass fraction) of the triglycerides C24 - C54 (even numbers) and cholesterol. The content of the triglycerides and of cholesterol has been normalised to 100 g. The relative mass fractions are presented in Table 1. The results of the certification exercise, which involved 11 European laboratories, are presented and discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) including uncertainties due to possible inhomogeneity and possible instability as well as uncertainty contributions from the calibrant (impurities and weighing).

Table 1: Certified values and expanded uncertainties for triglycerides C24 - C54 and cholesterol in pure butter fat (BCR-632A) and adulterated butter fat (BCR-632B).

Compound	BCR-632A			BCR-632B		
	Relative mass fraction in g/100 g[1)]		Number of accepted sets of results	Relative mass fraction in g/100 g[1)]		Number of accepted sets of results
	Certified value[2)]	Uncertainty[3)]		Certified value[2)]	Uncertainty[3)]	
C24	0.07	0.04	8	0.08	0.04	8
Cholesterol	0.289	0.012	8	0.278	0.011	8
C26	0.33	0.06	8	0.34	0.06	8
C28	0.74	0.07	8	0.75	0.06	8
C30	1.37	0.08	8	1.46	0.07	8
C32	2.83	0.14	8	3.30	0.12	8
C34	6.09	0.29	8	6.57	0.25	8
C36	10.7	0.5	8	11.1	0.4	8
C38	12.5	0.4	8	12.7	0.4	8
C40	10.05	0.19	8	10.07	0.17	8
C42	7.07	0.13	8	7.10	0.10	8
C44	6.68	0.12	8	6.57	0.12	8
C46	7.36	0.17	8	7.12	0.17	8
C48	8.74	0.21	8	8.42	0.19	8
C50	10.74	0.24	8	10.28	0.19	8
C52	9.8	0.4	8	9.36	0.28	8
C54	4.7	0.5	8	4.5	0.4	8

1) Sum of the triglycerides and of cholesterol has been normalised to 100 g.

2) Unweighted mean value of the means of accepted sets of results, each set being obtained in a different laboratory and/or with a different method of determination.

3) Estimated expanded uncertainty U_{CRM} with a coverage factor $k = 2$, corresponding to a level of confidence of about 95 %, as defined in the Guide to the Expression of Uncertainty in Measurement (GUM), ISO, 1993.

CONTENTS

GLOSSARY OF TERMS

ANOVA	analysis of variances
BCR	Community Bureau of Reference
BHA	butylated hydroxyanisole (= 2-*t*-butyl-4-methoxyphenol)
BHT	butylated hydroxytoluene (= 2,6-di-*t*-butyl-4-methylphenol)
CAP	Common Agricultural Policy
Cxx	triglyceride with the respective carbon number (number of all fatty acid carbon atoms excluding glycerol carbon atoms)
Chol.	cholesterol
CI	confidence interval
CRM	certified reference material
df	film thickness
DG AGRI	European Commission, Directorate General Agriculture
FAME	fatty acid methyl ester
FDA	United States Food and Drug Administration
FID	flame ionisation detector
GC	gas chromatography
GUM	Guide to the expression of Uncertainty in Measurement
HPLC	high performance liquid chromatography
IRMM	Institute for Reference Materials and Measurements (BE)
MeOH	methanol
MS	mass spectrometry
NIST	National Institute for Standards and Technology (US)
RF	response factor
RM	reference material
RSD	relative standard deviation
SD	standard deviation
SI	International System of Units
TBHQ	*t*-butyl-hydroquinone
TG	triglyceride
USDA	United States Department of Agriculture

1 INTRODUCTION

The adulteration of butter with less expensive foreign fats such as lard, palm kernel fat, rape seed, fish oil or beef tallow represents considerable fraud as it undermines the correct allocation of payments within the intervention buying scheme of the European Union [1]. This market management tool under the CAP shall maintain market prices during short-term fluctuations in supply. As butter is only bought in by intervention agencies meeting the requirements laid down in Article 6 of Regulation (EC) 1255/1999 [2], measures have to be taken to assure the identity and quality of the butter and to prevent fraudulent practices.

An efficient analytical methodology to ensure the authenticity of milk fat is the separation and quantification of its triglycerides by means of gas chromatography (GC) with flame ionisation detection (FID). The obtained patterns are specific for a given type of fat in terms of distribution of triglycerides and their relative quantities. Subsequently, these results are subjected to calculations using triglyceride formulae with so-called S-values [3–7], which allow the detection of foreign fat at a low percentage level. If these limits are transgressed, the presence of a foreign fat can be assumed.

Upon request of DG AGRI a new set consisting of two CRMs (pure and adulterated butter fat) was prepared and made available with the intention to increase the accuracy of analytical data in the respective EU testing laboratories and to facilitate comparability of data sets. For the laboratories, these CRMs provide a tool to either calibrate their method and/or to check the performance of their applied method.

2 PARTICIPANTS

- **Preparation of material**
  - S.A.N. Corman, Goé (BE)
  - EC, JRC, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

- **Homogeneity and stability studies**
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  - EC, JRC, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

- **Organisation, statistical analysis and preparation of the report**
  - EC, JRC, Institute for Reference Materials and Measurements (IRMM), Geel (BE)

3 PREPARATION OF MATERIALS

3.1 List of Compounds and Quantities Used

Anhydrous milk fat (Corman, industrial production, July 1996):

Raw material from the transition from grass to barn feeding period was selected. The following production steps have been carried out:

- Melting of butter
- Filtration
- Centrifugal separation of fat phase from water phase
- Vacuum evaporation of fat phase

Coconut oil (Vamo-Fuji, May 1996):

The refined coconut oil sample no. T741 was used.

Antioxidants:

The antioxidant TENOX 26 (Eastman Chemical Company), consisting of BHA, 10 %, BHT, 10 % and TBHQ, 6 %, was used.

3.2 Production

Pure butter fat:

32.0 kg of anhydrous milk fat and 24.32 g TENOX 26 (corresponding to a level of 0.076 g/100 g) were mixed in a 70 L vessel for 15 min using a propeller. Mixing was performed at 45 °C and under nitrogen atmosphere.

Adulterated butter fat:

30.4 kg anhydrous milk fat, 1.6 kg coconut oil and 24.32 g TENOX 26 (corresponding to a level of 0.076 g/100 g) were mixed in a 70 L vessel for 15 min using a propeller. Mixing was performed at 45 °C and under nitrogen atmosphere.

All the amounts were weighed in using calibrated balances (Mettler Toledo, CH).

The material produced by Corman was shipped to the IRMM in 4 batches (A and B, pure butter fat; C and D, adulterated butter fat), which have been characterised as follows (data provided by Corman):

Table 2: Analysis bulletin of produced butter fat batches.

Analysis	Units	Value batch A	Value batch B	Value batch C	Value batch D
Water content	g/100 g	0.06	0.06	0.06	0.07
Free fatty acids	g/100 g (calc. as oleic acid)	0.18	0.17	0.16	0.17
Peroxide value	meq. O_2/kg	0.04	0.03	0.03	0.04
Iron content	mg/kg	0.298	<0.01	<0.01	<0.01
Copper content	mg/kg	0.006	<0.005	<0.005	<0.005

3.3 Ampouling at IRMM

The materials as delivered from Corman were first stored at room temperature for 24 h and then placed at 50 °C in a drying oven to ensure complete melting of the butter. The butter fat was then transferred to a pre-warmed glass container, which was flushed with nitrogen before. Nitrogen was also used to homogenise the liquid butter fat. All tubing, needles and devices getting into contact with the liquid butter fat were also pre-warmed to prevent the fat to crystallise. For ampouling, 10 mL amber glass ampoules were used. Prior to filling, the ampoules were pre-flushed with a mixture of 90 % Ar / 10 % He. After filling of 5 mL butter fat per unit, the ampoules were flushed again with the inert gas mixture and sealed in a flame (torch-sealed). After labelling, the ampoules were stored at –30 °C.

For the pure butter fat, only batch B was used (A was possibly contaminated with iron). 3400 ampoules were produced under the conditions described above.

For the adulterated butter fat, batches C and D were mixed and 3380 ampoules were produced. The remaining butter fat was stored at –30 °C.

3.4 Component of CRM Uncertainty and Traceability

Uncertainties of the certified values were estimated according to the Guide to the Expression of Uncertainty in Measurements (GUM [8]). This required the estimation of the uncertainty of the characterisation (u_{char}), homogeneity (u_{bb}) and the stability (u_{lts}), as recently described by Pauwels et al. [9] and van der Veen et al. [10-12]. The uncertainty of the characterisation was derived from the certification measurements. An estimate of the heterogeneity was obtained from the homogeneity study. Furthermore, the calibrant composition uncertainty ($u_{calibrant}$) taking into account impurities of the components and weighing errors was included in the overall uncertainty.

The certified property values for BCR-632A and B are traceable to the method as described in Annex XXV of the Commission Regulation 213/2001 [7].

4 HOMOGENEITY STUDY

Homogeneity measurements were performed under repeatability conditions. 34 ampoules, taken out in regular intervals from the produced batch, were analysed in duplicate for each of the analytes.

The results were evaluated according to Linsinger et al. [13], by a one-way analysis of variance (ANOVA), which allows the separation of the method variation (s_{wb}) from the combined uncertainty of the between unit experiment ($u_{c,bb}$) to obtain an estimate for the real variation between units (s_{bb}) as shown in equation (1):

$$u_{c,bb}^2 = s_{bb}^2 + \frac{s_{wb}^2}{n} \qquad (1)$$

The standard deviation between the units was used as the estimator for the between-units variance. The measurement variation sets a lower limit u_{bb}^* to this estimator, which is given by equation (2)

$$u_{bb}^* = \sqrt{\frac{MS_{within}}{n}} \sqrt[4]{\frac{2}{v_{MSwithin}}} \qquad (2)$$

with MS_{within}, n and $v_{MSwithin}$ being the mean squares within units, the number of measurements per unit (2, duplicate analysis) and the degrees of freedom of MS_{within}, respectively. The uncertainty of homogeneity (u_{bb}) is consequently estimated as s_{bb} or u_{bb}^*, depending on which of these is larger. The statistical evaluation is summarised in Tables 3 and 4. A trend test was performed, which showed that there was no trend over the filling and the analytical sequence. More details of this homogeneity study can be found elsewhere [14].

Table 3: Data of the homogeneity evaluation of BCR-632A.
(Average values are given in mass fractions in g/100 g;
n.c. = not calculable because $MS_{among} < MS_{within}$)

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
Average	0.1033	0.2899	0.3179	0.7180	1.3496	2.8201	5.9572	10.0979	12.1462
s_{wb}	0.0084	0.0083	0.0060	0.0125	0.0120	0.0209	0.0348	0.0341	0.0253
s_{bb}	0.0015	n.c.	0.0016	n.c.	0.0014	n.c.	n.c.	n.c.	0.0037
u_{bb}^*	0.0029	0.0029	0.0021	0.0043	0.0042	0.0073	0.0121	0.0119	0.0088
u_{bb}	0.0029	0.0029	0.0021	0.0043	0.0042	0.0073	0.0121	0.0119	0.0088
u_{bb} [%]	2.8314	0.9913	0.6571	0.6049	0.3092	0.2580	0.2035	0.1176	0.0725

	C40	C42	C44	C46	C48	C50	C52	C54
Average	9.9851	7.1161	6.8102	7.5564	9.0430	10.9096	9.9858	4.7936
s_{wb}	0.0133	0.0153	0.0185	0.0216	0.0301	0.0388	0.0399	0.0261
s_{bb}	0.0071	n.c.	n.c.	n.c.	n.c.	n.c.	0.0074	0.0122
u_{bb}^*	0.0046	0.0053	0.0064	0.0075	0.0105	0.0135	0.0139	0.0091
u_{bb}	0.0071	0.0053	0.0064	0.0075	0.0105	0.0135	0.0139	0.0122
u_{bb} [%]	0.0710	0.0749	0.0945	0.0995	0.1157	0.1240	0.1392	0.2541

Table 4: Data of the homogeneity evaluation of BCR-632B.
(Average values are given in mass fractions in g/100 g;
n.c. = not calculable because $MS_{among} < MS_{within}$)

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
Average	0.0937	0.2671	0.3853	0.8205	1.5428	3.3307	6.3440	10.2755	12.0954
s_{wb}	0.0045	0.0034	0.0090	0.0131	0.0150	0.0173	0.0180	0.0306	0.0182
s_{bb}	0.0029	0.0009	0.0008	0.0042	0.0052	0.0083	0.0094	n.c.	n.c.
u^*_{bb}	0.0016	0.0012	0.0031	0.0045	0.0052	0.0060	0.0063	0.0106	0.0064
u_{bb}	0.0029	0.0012	0.0031	0.0045	0.0052	0.0083	0.0094	0.0106	0.0064
u_{bb} [%]	3.0709	0.4436	0.8124	0.5544	0.3378	0.2502	0.1482	0.1036	0.0525

	C40	C42	C44	C46	C48	C50	C52	C54
Average	9.9400	7.2560	6.8452	7.4879	8.8706	10.4730	9.4488	4.5237
s_{wb}	0.0104	0.0167	0.0197	0.0184	0.0149	0.0261	0.0419	0.0286
s_{bb}	0.0035	n.c.	n.c.	n.c.	n.c.	0.0144	0.0183	0.0119
u^*_{bb}	0.0036	0.0058	0.0069	0.0064	0.0052	0.0091	0.0146	0.0100
u_{bb}	0.0036	0.0058	0.0069	0.0064	0.0052	0.0144	0.0183	0.0119
u_{bb} [%]	0.0363	0.0800	0.1002	0.0857	0.0584	0.1379	0.1934	0.2633

5 STABILITY STUDY

Three studies have been carried out: a short-term stability study (0 to 12 weeks) to assess the conditions of transport and dispatch, and a mid-term study (0 to 12 months) as well as a long-term study (0 to 36 months). Isochronous measurement schemes [15] were employed, which allow the analysis of all samples of one study at the same time. Thus, the analytical error was reduced by applying repeatability rather than reproducibility conditions. The data for mid- and long-term stability studies were normalised and combined for the calculation of u_{lts}.

5.1 Short-Term Stability

Short-term stability was tested by an isochronous study with measurements after 0, 1, 2 and 4 weeks as well as 12 weeks at 40 °C. The results were subjected to regression analysis. The slopes of the regression functions were tested for significance. Additionally, the results after 0 and 1 week at 60 °C were compared by a t-test (two-sided, equal variances). A summary of these results is shown in Tables 5 and 6. Eight replicates were performed for t = 0 and 6 for all other storage time points.

Table 5: Results of the short-term stability study of BCR-632A.

	C24	**Chol.**	**C26**	**C28**	**C30**	**C32**	**C34**	**C36**	**C38**
Slope 40 °C [%/week]	-0.38	-0.07	-0.11	-0.04	-0.03	0.00	0.00	0.01	0.01
Test of significance of the slope at 40 °C (95 %)	no	no	no	no	no	no	no	no	no
Slope 60 °C [%/week]	11.38	0.34	2.85	1.26	0.74	0.52	0.40	0.41	0.16
Test of significance of the slope at 60 °C (95 %)	no	no	no	no	no	no	no	no	no

	C40	**C42**	**C44**	**C46**	**C48**	**C50**	**C52**	**C54**
Slope 40 °C [%/week]	0.01	0.01	0.01	0.01	0.00	-0.01	-0.01	-0.03
Test of significance of the slope at 40 °C (95 %)	no	no	no	no	no	no	no	no
Slope 60 °C [%/week]	0.10	0.07	-0.03	-0.26	-0.46	-0.37	-0.41	-0.35
Test of significance of the slope at 60 °C (95 %)	no	no	no	no	no	no	no	no

Table 6: Results of the short-term stability study of BCR-632B.

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
Slope 40 °C [%/week]	-0.21	-0.04	0.00	0.01	0.00	-0.01	-0.02	-0.02	-0.01
Test of significance of the slope at 40 °C (95 %)	no	no	no	no	no	no	no	no	no
Slope 60 °C [%/week]	1.61	5.70	5.34	4.05	2.95	2.27	1.33	0.72	0.41
Test of significance of the slope at 60 °C (95 %)	no	yes	no	yes	yes	yes	yes	yes	no

	C40	C42	C44	C46	C48	C50	C52	C54
Slope 40 °C [%/week]	-0.01	0.00	0.00	0.01	0.02	0.02	0.02	0.00
Test of significance of the slope at 40 °C (95 %)	no	no	no	no	no	no	no	no
Slope 60 °C [%/week]	0.16	0.08	-0.13	-0.19	-0.57	-0.96	-1.36	-2.65
Test of significance of the slope at 60 °C (95 %)	no	no	no	no	yes	yes	yes	yes

It can be concluded from the data in Tables 5 and 6 that normal dispatch at ambient temperature is sufficient as for both materials no significant degradation could be detected at 40 °C, even taking values for 3 months into account. However, for 60 °C data revealed a certain indication for a degradation of some triglycerides in the adulterated butter, whereas no significant degradation could be detected in the pure butter fat. More details of this short-term stability study can be found elsewhere [16].

5.2 Mid- and Long-Term Stability

For the mid-term stability study, measurements after 1, 2 and 4 weeks as well as after 3, 8 and 12 months at –20, 4 and 20 °C (reference temperature: -70 °C) were performed. Six measurements were performed at t = 0 for BCR-632A and –632B, respectively, whereas 3 replicates were analysed for the other time points at each temperature level. All values were normalised to the –70 °C results (cf. Tables 7 and 8).

The long-term stability study of both materials was evaluated after receiving data of tests after 12, 24 and 36 months, respectively. Twelve replicates at reference temperature (-70 °C) were measured, whereas 6 replicates at temperatures of –20, 4 and 20 °C were measured and all obtained values were normalised to the –70 °C results.

The two data sets were normalised to their respective mean (mean of reference samples; set to 1.00) and combined to one large data set. Regression lines were calculated, the slopes were tested for significance and, if not significant, u_{lts} for 2, 4 and 6 years were calculated.

The overall stability data for BCR-632A show none, 4 and 3 significantly unstable triglycerides for –20, 4 and 20 °C, respectively. Whereas for BCR-632B only 2 compounds exhibited significant values at 20 °C. For BCR-632A, the slopes of C36, C40, and C48 were found to be significant for 4 °C, whereas no significant difference from zero was found for

temperatures of –20 °C and 20 °C, respectively. Therefore, the obtained results had to be regarded as purely statistical and standard errors were calculated from the values of 4 °C and used in the calculation of the overall uncertainty. The same procedure was applied for C42, although significant slopes were observed for both 4 °C and 20 °C. For cholesterol and C46 as well as for C24 and cholesterol in BCR-632B, significant slopes were obtained for 20 °C, indicating some degradation of these compounds at room temperature on mid- or long-term storage. Therefore, the material is stored at 4 °C.

The values for 4 °C and 6 years (u_{lts}) were taken to calculate the overall uncertainty. Therefore, the resulting certificate is valid for 6 years. This validity can be prolonged if additional stability data are obtained. More details of the long-term stability study can be found elsewhere [17].

Table 7: Results of the stability evaluation of BCR-632A.
(u(b) = standard error of the slope [%/year];
u_{lts} (6 years) is obtained by multiplication of u(b) by 6)

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
–20 °C	***Slope sig. (95 %)**	no	no	no	no	no	no	no	no	no
	***u(b)* [%/year]**	1.49	0.36	1.01	0.64	0.41	0.36	0.34	0.25	0.10
	u_{lts} (6 years) [%]	8.94	2.16	6.06	3.84	2.46	2.16	2.04	1.50	0.60

		C40	C42	C44	C46	C48	C50	C52	C54
–20 °C	***Slope sig. (95 %)**	no	no	no	no	no	no	no	no
	***u(b)* [%/year]**	0.05	0.11	0.06	0.08	0.08	0.16	0.26	0.68
	u_{lts} (6 years) [%]	0.30	0.66	0.36	0.48	0.48	0.96	1.56	4.08

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
4 °C	***Slope sig. (95 %)**	no	no	no	no	no	no	no	yes	no
	***u(b)* [%/year]**	1.59	0.36	0.93	0.57	0.35	0.35	0.36	0.28	0.11
	u_{lts} (6 years) [%]	9.54	2.16	5.58	3.42	2.10	2.10	2.16	1.68	0.66

		C40	C42	C44	C46	C48	C50	C52	C54
4 °C	***Slope sig. (95 %)**	yes	yes	no	no	yes	no	no	no
	***u(b)* [%/year]**	0.07	0.13	0.05	0.09	0.09	0.16	0.24	0.72
	u_{lts} (6 years) [%]	0.42	0.78	0.30	0.54	0.54	0.96	1.44	4.32

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
20 °C	***Slope sig. (95 %)**	no	yes	no	no	no	no	no	no	no
	***u(b)* [%/year]**	1.80	0.35	0.89	0.56	0.36	0.33	0.35	0.25	0.10
	u_{lts} (6 years) [%]	10.80	2.10	5.34	3.36	2.16	1.98	2.10	1.50	0.60

		C40	C42	C44	C46	C48	C50	C52	C54
20 °C	***Slope sig. (95 %)**	no	yes	no	yes	no	no	no	no
	***u(b)* [%/year]**	0.06	0.14	0.05	0.09	0.09	0.15	0.23	0.72
	u_{lts} (6 years) [%]	0.36	0.84	0.30	0.54	0.54	0.90	1.38	4.32

**Test of significance of the slope.*

Table 8: Results of the stability evaluation of BCR-632B.
(u(b) = standard error of the slope [%/year];
u_{lts} (6 years) is obtained by multiplication of u(b) by 6)

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
–20 °C	*Slope sig. (95 %)	no	no	no	no	no	no	no	no	no
	u(b) [%/year]	1.04	0.34	0.62	0.39	0.35	0.45	0.39	0.28	0.08
	u_{lts} (6 years) [%]	6.24	2.04	3.72	2.34	2.10	2.70	2.34	1.68	0.48

		C40	C42	C44	C46	C48	C50	C52	C54
–20 °C	*Slope sig. (95 %)	no	no	no	no	no	no	no	no
	u(b) [%/year]	0.07	0.15	0.06	0.08	0.10	0.23	0.23	0.60
	u_{lts} (6 years) [%]	0.42	0.90	0.36	0.48	0.60	1.38	1.38	3.60

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
4 °C	*Slope sig. (95 %)	no	no	no	no	no	no	no	no	no
	u(b) [%/year]	0.86	0.28	0.51	0.31	0.23	0.24	0.27	0.20	0.07
	u_{lts} (6 years) [%]	5.16	1.68	3.06	1.86	1.38	1.44	1.62	1.20	0.42

		C40	C42	C44	C46	C48	C50	C52	C54
4 °C	*Slope sig. (95 %)	no	no	no	no	no	no	no	no
	u(b) [%/year]	0.05	0.09	0.04	0.07	0.06	0.11	0.19	0.54
	u_{lts} (6 years) [%]	0.30	0.54	0.24	0.42	0.36	0.66	1.14	3.24

		C24	Chol.	C26	C28	C30	C32	C34	C36	C38
20 °C	*Slope sig. (95 %)	yes	yes	no	no	no	no	no	no	no
	u(b) [%/year]	0.97	0.35	0.57	0.33	0.27	0.25	0.27	0.21	0.08
	u_{lts} (6 years) [%]	5.82	2.10	3.42	1.98	1.62	1.50	1.62	1.26	0.48

		C40	C42	C44	C46	C48	C50	C52	C54
20 °C	*Slope sig. (95 %)	no	no	no	no	no	no	no	no
	u(b) [%/year]	0.04	0.09	0.05	0.06	0.08	0.13	0.20	0.54
	u_{lts} (6 years) [%]	0.24	0.54	0.30	0.36	0.48	0.78	1.20	3.24

*Test of significance of the slope.

6 ANALYTICAL METHODS

A reference method (EU Regulation 454/95, Annex III and EU Regulation 213/2001, Annex XXV [4, 7]) describes the analysis of triglycerides in milk fat by GC-FID. TGs with odd acyl-The odd acyl-carbon numbers (2n + 1) are combined with the preceding even-numbered TG (2n).

The butter fat is diluted in *n*-heptane to a 5 % solution and applied onto a packed OV-1 column (100 % dimethyl polysiloxane) using a hot-injection technique [4]; the triglycerides are eluted from the column in the order of increasing molecular weight, corresponding to the increasing number of carbon atoms per triglyceride. This methodology has repeatedly demonstrated its suitability [18, 19].

However, the Regulation also allows the application of suitable capillary columns alternatively to the described packed column. Molkentin and Precht [20] have shown that a short capillary column coated with a high-temperature resistant stationary phase and employing cool on-column injection, renders similar results to a packed column in terms of resolution and relative quantities of triglycerides. Another ring trial [21] has shown that methods based on packed and on capillary columns are equally well applicable, despite some differences in reproducibility data.

The laboratories participating in this certification had to follow the Regulation; in contrast to the last certification exercise of BCR-519, finalised 1997, where 9 out of 10 participating laboratories used a packed column system, more than 50 % of the laboratories in this certification campaign employed capillary GC methods, which also reflects the development in modern GC towards capillary column based separation techniques. The length of the capillary had to be adjusted accordingly to obtain similar resolution as for the packed column; this was an important prerequisite for accurate peak assignment and integration.

7 CHARACTERISATION

7.1 Calibration Solution

The following chemicals and reagents were used:

Cholesterol, glyceryl trioctanoate (trioctanoin; C24), glyceryl tricaprinate (tricaprin; C30), glyceryl trilaurate (trilaurin; C36), glyceryl trimyristate (trimyristin; C42), glyceryl tripalmitate (tripalmitin; C48) and glyceryl tristearate (tristearin; C54) were obtained from Nu-Chek-Prep, Elysian (US). 1,2-Distearoyl-3-myristoyl-*rac*-glycerol (C50) and 1,2-distearoyl-3-palmitoyl-*rac*-glycerol (C52) were from Sigma, Bornem (BE). *n*-Heptane was from Merck, Darmstadt (DE).

A calibration solution consisting of cholesterol, C24, C30, C36, C42, C48, C50, C52 and C54 was prepared at IRMM and distributed to the participants for determining the response factors of the individual triglycerides prior to the certification measurements.

The uncertainty of the calibrant comprises contributions deriving from impurities of the compounds and weighing errors. The procedure of how to establish this uncertainty is described as follows:

Two laboratories were asked to perform purity determinations. This was accomplished by providing them with aliquots of the individual standard components and using the laboratory-specific GC-FID methods according to the EU Regulation [4].

Table 9: Purity determinations of triglycerides and cholesterol used in the preparation of the calibrant solution given in mass fractions in g/100 g.

Laboratory A									
C24	**Chol**	**C30**	**C36**	**C42**	**C48**	**C50**	**C52**	**C54**	**Imp.[1)]**
100									**C24**
	99.62								**Chol.**
		100							**C30**
			99.96						**C36**
				99.84					**C42**
					98.90	0.59			**C48**
						98.03	0.36		**C50**
							98.51		**C52**
							0.54	97.39	**C54**
Laboratory B									
C24	**Chol**	**C30**	**C36**	**C42**	**C48**	**C50**	**C52**	**C54**	**Imp.[1)]**
99.92									**C24**
	99.95								**Chol.**
		99.56							**C30**
			99.72						**C36**
				99.78					**C42**
					98.62	0.12			**C48**
					0.61	98.26	0.21		**C50**
						0.43	98.82	0.68	**C52**
							0.36	98.93	**C54**

[1)] *Contained impurity with retention time of indicated triglyceride.*

The purity was calculated as the ratio of the peak areas obtained for an identified compound to the total area under the peaks present in the chromatogram. In the case, impurities were detected, which matched in retention time with other triglycerides of the exercise, these had to be included in the respective fraction.

Table 9 shows the results of the purity determinations of the individual compounds. Blank runs injecting *n*-heptane proved the absence of any impurities, which might interfere with TG analysis. For instance, the purchased C50 contained also 0.59 g/100 g C48 (according the analytical results of laboratory A). Subsequently, the weighed quantities of triglycerides were corrected for the impurities with the same retention time. Impurities not matching retention times of triglycerides were not taken into account in terms of correcting for other fractions. In Table 10 the weighed amount of the individual compounds of the calibrant can be seen as well as the corrected masses and the mass fractions based on the mean of the impurity data of laboratory A and B.

Table 10: Composition of calibrant and corrected mass fractions

Triglyceride	Amount weighed in mg	Corrected mass in mg			Mass fraction in g/100 g
		Lab. A	Lab. B	Mean	
C24	1.57	1.57	1.57	1.57	0.11
Chol.	10.75	10.71	10.74	10.73	0.72
C30	37.31	37.31	37.15	37.23	2.49
C36	292.80	292.68	291.98	292.33	19.59
C42	186.80	186.50	186.39	186.45	12.49
C48	231.40	230.68	228.58	229.63	15.38
C50	311.10	306.03	307.71	306.87	20.56
C52	290.00	285.68	288.87	287.27	19.25
C54	141.80	139.66	141.33	140.50	9.41
SUM	**1503.53**	**1490.82**	**1494.32**	**1492.58**	**100**

The combined uncertainty of the calibrant (including impurity and weighing contribution) is shown in Table 11. The weighing error was determined to be ±0.2 mg, the uncertainty from the purity determination was calculated as half of the difference between the two laboratories related to the mean purity. For those components not present in the standard, the average of the relative combined uncertainties of C30 - C54 was used to calculate the final calibrant uncertainty (= 0.39 %). Further uncertainty contributions (e.g. from interpolation) were considered to be negligible.

Table 11: Calculation of the calibrant uncertainty (including weighing error and impurities).

TG	Weighing uncertainty in %	Impurity uncertainty in %	Combined uncertainty in %[1)]
C24	12.739	0.040	12.74
Chol.	1.860	0.165	1.87
C30	0.536	0.220	0.58
C36	0.068	0.120	0.14
C42	0.107	0.030	0.11
C48	0.086	0.452	0.46
C50	0.064	0.266	0.27
C52	0.069	0.545	0.55
C54	0.141	0.585	0.60

[1)] *Rounded to 2 decimals.*

7.2 Characterisation Measurements

Each laboratory was provided with 1 ampoule each of BCR-632A and B. They were asked to perform 6 independent analyses on 2 measurement days (calibration of the system on each measurement day). The laboratories applied their in-house method, which complied with the reference method [4, 7]. Both, capillary and packed column–based methods were used (see Annex, Table A-1). Calibration of the systems was accomplished by using the calibration solution provided by IRMM. Data evaluation and re-calculation if applicable was carried out at IRMM. Tables 12 and 13 list the summary of the results.

Table 12: Statistical evaluation of BCR-632A certification interlaboratory exercise.
(11 laboratories submitted 8 accepted sets of results)

	C24	**Chol.**	**C26**	**C28**	**C30**	**C32**	**C34**	**C36**	**C38**
Overall average [mass fraction in g/100 g]	0.07	0.29	0.33	0.74	1.37	2.83	6.09	10.67	12.52
s of laboratory averages [mass fraction in g/100 g]	0.04	0.02	0.06	0.07	0.07	0.08	0.14	0.35	0.37
Std. error of overall average[1)] [mass fraction in g/100 g]	0.01	0.01	0.02	0.02	0.02	0.03	0.05	0.12	0.13
Relative std. error [in %]	17.60	2.51	6.62	3.29	1.82	0.98	0.79	1.16	1.05
s within laboratories[2)] [mass fraction in g/100 g]	0.01	0.01	0.01	0.01	0.01	0.02	0.04	0.06	0.04
s between laboratories[2)] [mass fraction in g/100 g]	0.04	0.02	0.06	0.07	0.07	0.08	0.13	0.35	0.37

	C40	**C42**	**C44**	**C46**	**C48**	**C50**	**C52**	**C54**
Overall average [mass fraction in g/100 g]	10.05	7.07	6.68	7.36	8.74	10.74	9.78	4.68
s of laboratory averages [mass fraction in g/100 g]	0.20	0.07	0.13	0.19	0.23	0.14	0.22	0.19
Std. error of overall average[1)] [mass fraction in g/100 g]	0.07	0.02	0.05	0.07	0.08	0.05	0.08	0.07
Relative std. error [in %]	0.70	0.34	0.71	0.92	0.91	0.46	0.79	1.45
s within laboratories[2)] [mass fraction in g/100 g]	0.07	0.10	0.06	0.04	0.05	0.08	0.08	0.05
s between laboratories[2)] [mass fraction in g/100 g]	0.20	0.06	0.13	0.19	0.23	0.14	0.22	0.19

[1)] *Standard error = $s/\sqrt{n}$*

[2)] *Data derived from ANOVA*

Table 13: Statistical evaluation of BCR-632B certification interlaboratory exercise.
(11 laboratories submitted 8 accepted sets of results)

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
Overall average [mass fraction in g/100 g]	0.08	0.28	0.34	0.75	1.46	3.30	6.57	11.05	12.73
s of laboratory averages [mass fraction in g/100 g]	0.03	0.02	0.07	0.07	0.07	0.08	0.15	0.37	0.39
Std. error of overall average[1)] [mass fraction in g/100 g]	0.01	0.01	0.02	0.03	0.03	0.03	0.05	0.13	0.14
Relative std. error [in %]	15.21	2.72	6.72	3.34	1.80	0.89	0.78	1.17	1.09
s within laboratories[2)] [mass fraction in g/100 g]	0.01	0.01	0.01	0.01	0.02	0.02	0.04	0.07	0.05
s between laboratories[2)] [mass fraction in g/100 g]	0.02	0.02	0.07	0.07	0.07	0.08	0.15	0.36	0.39

	C40	C42	C44	C46	C48	C50	C52	C54
Overall average [mass fraction in g/100 g]	10.07	7.10	6.57	7.12	8.42	10.28	9.36	4.51
s of laboratory averages [mass fraction in g/100 g]	0.20	0.07	0.14	0.20	0.23	0.17	0.20	0.20
Std. error of overall average[1)] [mass fraction in g/100 g]	0.07	0.03	0.05	0.07	0.08	0.06	0.07	0.07
Relative std. error [in %]	0.69	0.37	0.77	0.99	0.94	0.57	0.75	1.59
s within laboratories[2)] [mass fraction in g/100 g]	0.08	0.09	0.06	0.04	0.05	0.06	0.08	0.08
s between laboratories[2)] [mass fraction in g/100 g]	0.19	0.06	0.14	0.20	0.22	0.16	0.20	0.20

[1)] *Standard error = $s/\sqrt{n}$*

[2)] *Data derived from ANOVA*

7.3 Technical Evaluation of the Results

Results were eliminated only, if there were indications of technical problems, but not on purely statistical reasons.

The data sets of laboratories 9 to 11 were withdrawn due to substantial calibration problems. Laboratory 9 indicated only one common response factor (RF) for C24 and cholesterol due to the reported insufficient resolution of these two components; consequently only a sum value was indicated for C24 and cholesterol. Furthermore, for C26 and C28 the same RF was taken as determined for C30. Laboratory 10 reported substantial difficulties in establishing a reproducible RF value for C24 (deviations of ±50 % and more upon repeated injections). Therefore, the RF was arbitrarily set to 1.00. Furthermore, for C26 and C28 the same RF value was taken as determined for C30. Laboratory 11 had persistent difficulties to obtain meaningful RFs. Therefore, the existing butter fat reference material BCR-519 [6] was used for calibration. This procedure was not accepted as the calibration of a method to establish certified values for a new certified reference material had to be independent from any former material.

7.3.1 Calculations

The calculation of triglyceride RFs of compounds not present in the calibrant is a prerequisite for calculating the relative mass fractions, which in turn are required to determine the S-values using the triglyceride formulae [4, 7]. The minor butter fat component C24 is not utilised in any of the triglyceride formulae. Therefore, its value influences the relative mass fractions of the other triglycerides upon normalisation only to a very minor extent.

The laboratories in this exercise employed different procedures for calculating the RFs of triglycerides not present in the calibrant. One commonly used practice is to extrapolate from C30 to obtain values for C26 and C28 in case the measured value for C24 substantially differs from 1.00. Other practices include arbitrary setting of the RF for C24 to 1.00, if no values close to the theoretical one are found. Furthermore, various statistical functions (least square model, 3rd order curve, etc.) are applied to obtain RFs for components not present in the standard.

To standardise the procedure, the obtained data were processed when necessary as follows:

All analysed RFs were taken into account; this implies that values substantially differing from 1.00 were kept unless there was clear evidence of technical problems such as insufficient resolution. Subsequently, strict linear interpolation was applied to obtain RFs for components not present in the calibrant. Upon calculating the respective relative mass fractions of the components in g/100 g, the data were normalised to 100 g (sum of triglycerides C24 - C54 and cholesterol).

In the following the necessary recalculations are described for each laboratory.

For laboratory 1, the submitted data had to be first normalised to 100 g (total content of triglycerides C24 - C54 and cholesterol), then the 3rd order curve utilised by the laboratory was replaced by full arithmetic interpolation. Laboratory 5 performed 6 runs per day of each material. The first 3 runs were taken into account (repeatability in range of 0.4 – 2.2 % between the runs). The applied least-square model was replaced by the arithmetic interpolation approach. Laboratory 4 has analysed 2 replicates of each sample; because of a high precision (0.2 – 2.1 % between replicates) only the area % values of one of the two replicates were taken into account. RFs for C26 and C28 were obtained by extrapolation from C30. Furthermore, RFs were calculated on a monthly basis by analysing a larger series of calibration samples and taking the respective mean values. This was replaced by considering the obtained calibration data using the IRMM calibrant on the two measurement days and strict arithmetic interpolation for all TG-RFs. For laboratories 6 and 8 minor changes had to be done (changing extrapolation from C30 to C26 and C28 to strict interpolation and replacing the curve-fit model by strict interpolation, which resulted in very minor differences).

7.3.2 Calibration solution

The calibration solution (total TG content of 2.5 % (w/w)) tends to precipitate after a few hours at room temperature, whereas in contrast, a 5 % (w/w) solution of butter fat in *n*-heptane does not show any noticeable precipitation under the same conditions. This problem could be circumvented by warming the calibrant to approx. 40 °C and by shortening the time towards injection as much as possible. However, for larger sample series special arrangements had to be foreseen such as a temperature-controlled auto-sampler. In capillary-based methods only approx. 5 – 10 % of the amount needed for a packed column analysis is required. Thus a simple dilution of the calibrant down to 0.25 – 1 % basically eliminates any precipitation problems.

Another critical issue about the calibrant is its composition (relative amounts of TGs). The calibrant used during this certification exercise comprised relative concentrations of TGs close to those found in butter fat, which has the advantage that the same conditions applied for the calibrant and the samples in terms of resolution between C24 and cholesterol as well as resolution between C48 - C50, C50 – C52 and C52 – C54.

7.3.3 Chromatographic data (comparison and technical observations)

Six out of 11 laboratories in this certification exercise utilised capillary-column methods. Capillaries from different suppliers with different lengths, diameters and film thicknesses (df) of coating were used (see Annex A, Table A-1). Furthermore, the methods differed in temperature programmes (gradients), carrier gases used, composition of the FID gases, injection times (data not shown) and injected amounts of the calibrant and the butter fat.

For 14 out of 17 compounds, smaller within-group standard deviations were found for the capillary column data compared to the packed column data set. This is remarkable as the applied capillary methods differ to a considerably larger extent from one another (column length, coating, dimensions, temperature programme, carrier gas, etc.) than the more consistent packed column methods. These results demonstrate that the applied capillary column methods are at least equally well applicable for this analysis.

8 CERTIFIED UNCERTAINTIES AND CERTIFIED VALUES

The uncertainty contributions to the certified values of a CRM [9] can be written as (3):

$$U_{CRM} = k * \sqrt{u_{char}^2 + u_{bb}^2 + u_{lts}^2 + u_{sts}^2 + u_{others}^2} \qquad (3)$$

U_{CRM} = *expanded uncertainty contribution to the certified value of a CRM*
k = *coverage factor (k = 2)*
u_{char} = *uncertainty of the certified property of the batch (characterisation)*
u_{bb} = *uncertainty contribution of between-bottle inhomogeneity*
u_{lts} = *uncertainty contribution of long-term stability (storage)*
u_{sts} = *uncertainty contribution of short-term stability (transport)*
u_{others} = *other uncertainty sources (incl. $u_{calibrant}$)*

The standard error of the laboratory means was used as estimation of the uncertainty of the concentration of the batch. The standard deviations between bottles were used as estimation of the uncertainty due to possible inhomogeneity, as explained in Section 4. u_{sts} was assumed to be negligible, as the material will be shipped at ambient temperature and no degradation is expected to happen during this short time. The estimation of u_{lts} was derived from regression analysis of the combined mid-term and long-term stability study. The standard deviation of the slope (under assumption of a slope of zero) was calculated. Six years were chosen as a proper time of validity of the certificate. The validity can be extended, if further stability tests show no significant degradation. The standard deviation of the slope was multiplied with this time to give the uncertainty due to possible instability. A coverage factor of 2 was applied to obtain expanded uncertainties. The individual uncertainty components, the combined standard uncertainties and the expanded uncertainties are shown in Tables 14 and 15. These certified values are valid until 7/2006.

Table 14: Calculation of expanded uncertainties for the triglycerides C24 – C54 and cholesterol in BCR-632A.
(Coverage factor k = 2)

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
u_{char} [%]	18.19	2.51	6.62	3.29	1.83	0.98	0.79	1.16	1.05
u_{bb} [%]	2.83	0.99	0.66	0.60	0.31	0.26	0.20	0.12	0.07
u_{lts} [%]; 6 years; 4 °C	9.54	2.16	5.58	3.42	2.10	2.10	2.16	1.68	0.66
$u_{calibrant}$ [%]	12.74	1.87	0.39	0.39	0.58	0.39	0.39	0.14	0.39
Combined uncertainty [%]	24.33	3.93	8.69	4.80	2.86	2.36	2.34	2.05	1.30
Certified value [g/100 g]	**0.07**	**0.289**	**0.33**	**0.74**	**1.37**	**2.83**	**6.09**	**10.7**	**12.5**
Combined uncertainty [g/100 g]	0.018	0.006	0.029	0.04	0.04	0.07	0.15	0.22	0.17
Expanded combined uncert. [g/ 100 g]	**0.04**	**0.012**	**0.06**	**0.07**	**0.08**	**0.14**	**0.29**	**0.5**	**0.4**

	C40	C42	C44	C46	C48	C50	C52	C54
u_{char} [%]	0.70	0.34	0.71	0.92	0.91	0.46	0.79	1.45
u_{bb} [%]	0.07	0.07	0.09	0.10	0.12	0.12	0.14	0.25
u_{lts} [%]; 6 years; 4 °C	0.42	0.78	0.30	0.54	0.54	0.96	1.44	4.32
$u_{calibrant}$ [%]	0.39	0.11	0.39	0.39	0.46	0.27	0.55	0.60
Combined uncertainty [%]	0.91	0.86	0.87	1.14	1.16	1.11	1.74	4.60
Certified value [g/100 g]	**10.05**	**7.07**	**6.68**	**7.36**	**8.74**	**10.74**	**9.8**	**4.7**
Combined uncertainty [g/100 g]	0.10	0.07	0.06	0.09	0.11	0.12	0.17	0.22
Expanded combined uncert. [g/100 g]	**0.19**	**0.13**	**0.12**	**0.17**	**0.21**	**0.24**	**0.4**	**0.5**

Table 15: Calculation of expanded uncertainties for the triglycerides C24 – C54 and cholesterol in BCR-632B.
(Coverage factor k = 2)

	C24	Chol.	C26	C28	C30	C32	C34	C36	C38
u_{char} [%]	15.76	2.72	6.72	3.34	1.80	0.89	0.78	1.17	1.09
u_{bb} [%]	3.07	0.44	0.81	0.55	0.34	0.25	0.15	0.10	0.05
u_{lts} [%]; 6 years; 4 °C	5.16	1.68	3.06	1.86	1.38	1.44	1.62	1.20	0.42
$u_{calibrant}$ [%]	12.74	1.87	0.39	0.39	0.58	0.39	0.39	0.14	0.39
Combined uncertainty [%]	21.14	3.73	7.44	3.89	2.36	1.76	1.85	1.68	1.23
Certified value [g/100 g]	**0.08**	**0.278**	**0.34**	**0.75**	**1.46**	**3.30**	**6.57**	**11.1**	**12.7**
Combined uncertainty [g/100 g]	0.017	0.006	0.026	0.030	0.04	0.06	0.13	0.19	0.16
Expanded combined uncert. [g/ 100 g]	**0.04**	**0.011**	**0.06**	**0.06**	**0.07**	**0.12**	**0.25**	**0.4**	**0.4**

	C40	C42	C44	C46	C48	C50	C52	C54
u_{char} [%]	0.69	0.37	0.77	0.99	0.94	0.57	0.75	1.59
u_{bb} [%]	0.04	0.08	0.10	0.09	0.06	0.14	0.19	0.26
u_{lts} [%]; 6 years; 4 °C	0.30	0.54	0.24	0.42	0.36	0.66	1.14	3.24
$u_{calibrant}$ [%]	0.39	0.11	0.39	0.39	0.46	0.27	0.55	0.60
Combined uncertainty [%]	0.85	0.67	0.90	1.15	1.11	0.93	1.49	3.67
Certified value [g/100 g]	**10.07**	**7.10**	**6.57**	**7.12**	**8.42**	**10.28**	**9.36**	**4.5**
Combined uncertainty [g/100 g]	0.09	0.05	0.06	0.09	0.10	0.10	0.14	0.17
Expanded combined uncert. [g/100 g]	**0.17**	**0.10**	**0.12**	**0.17**	**0.19**	**0.19**	**0.28**	**0.4**

9 DESCRIPTION OF MATERIAL, STORAGE AND INSTRUCTIONS FOR USE

9.1 Description of the Materials and Storage

BCR-632A and B are supplied in units of about 5 g each in amber glass ampoules, which were filled under inert gas conditions (argon / helium = 90 % / 10). The samples must be stored unopened and refrigerated at 4 °C or lower until use. The material is solid at ambient temperature.

9.2 Preparation and Use of the Materials and Minimum Sample Intake

Before opening an ampoule and taking out a portion for analysis, it must be guaranteed that the content is a liquid, and that it is properly mixed to ensure homogeneity. The following procedure is recommended:

1) Immerse the ampoule in a water bath at a temperature not exceeding 50 °C, with occasional agitation until the fat is completely melted.
2) When a clear bright oil is obtained, mix the contents by repeated inversion for at least 30 s.
3) Before significant cooling can occur, score the neck of the ampoule at the thinnest part of its head and remove the head manually or by application of a respective device.
4) Immediately transfer the contents of the ampoule in a clean and dry vial, which can be tightly sealed.

The reference material should be used on the day of opening. The contents of opened ampoules should not be stored for future use.

Minimum recommended sample intakes:
The reference methods define the amount of sample to be taken for the individual parameters.

9.3 Use of the Certified Reference Values

The certified values of BCR-632A and B are method-specific [4, 7]. They may be employed in two ways:

a) To check the validity of a calibration curve. In this case the value to be used is the certified mean value with the corresponding uncertainty.
b) To check the performance of a method. In this case, the user shall demonstrate that the repeatability of the method is compatible with the repeatabilities of the certifying laboratories.

For the latter purpose, the users can check whether the mean of their results (y) lies within the limits (4):

$$(\text{certified value} - 2\,\sigma) < y < (\text{certified value} + 2\,\sigma) \qquad (4)$$

For σ, either the long-term laboratory reproducibility (control chart) or the standard deviation between laboratories from the certification collaborative study is used.

10 REFERENCES

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11 ANNEX A (Instruments and Methods)

Table A-1: Gas chromatographic conditions for the analysis of triglycerides and cholesterol in pure and adulterated butter fat.

Lab. no.	Column type / dimensions	Carrier gas / flow rate (press.)	Injector / detector	Injection vol. & quantity	Temperature programme
1	BPX5 (SGE) 12 m x 0.53 mm df = 0.25 µm	He 14 mL/min	cool on-column ramp as oven temperature FID 370 °C	calibrant 1 µL (0.5 µg) sample 1 µL (1 µg)	70 °C (hold 0.5 min) to 190 °C with 50 °C/min to 350 °C with 6 °C/min (hold 20 min)
2	HT Ultimetal Simdist CB (Chrompack) 5 m x 0.53 mm df = 0.15 µm	N_2 15 kPa	cool on-column (80 °C) FID 350 °C	calibrant 0.5 µL (5 µg) sample 0.5 µL (5 µg)	80 °C (hold 1 min) to 190 °C with 50 °C/min to 350 °C with 6 °C/min (hold 6 min)
3	HT Ultimetal Simdist DB (Chrompack) 5 m x 0.53 mm df = 0.17 µm	He 14 mL/min	cool on-column (73 °C) FID 370 °C	calibrant 0.5 µL (2.5 µg) sample 0.5 µL (2.5 µg)	70 °C (hold 1 min) to 210 °C with 40 °C/min to 350 °C with 6 °C/min (hold 5 min)
4	SE52 (MEGA Carbo Erba) 5 m x 0.32 mm df = 0.15 µm	He 80 kPa	cool on-column (80 °C), FID 350 °C	calibrant 1 µL (2.5 µg) sample 1 µL (3 µg)	80 °C (hold 0.5 min) to 350 °C with 25 °C/min (hold 5 min)
5	3 % OV1 on Gaschrom Q (Chrompack) 0.6 m x 2.2 mm df = 125/150 µm	N_2 40 mL/min	injector 370 °C FID 370 °C	calibrant 1 µL (25 µg) sample 0.5 µL (25 µg)	210 °C (hold 1 min) to 350 °C with 6 °C/min (hold 5 min)
6	3 % OV1 on Gaschrom Q (Chrompack) 0.5 m x 2 mm df = 125/150 µm	N_2 40 mL/min	injector 370 °C FID 370 °C	calibrant 1 µL (25 µg) sample 1 µL (50 µg)	210 °C (hold 1 min) to 350 °C with 6 °C/min (hold 4 min)
7	3 % OV1 on Gaschrom Q (Chrompack) 0.6 m x 2 mm df = 125/150 µm	He 30.1 mL/min	injector 370° C FID 370° C	calibrant 0.5 µL (25 µg) sample 0.5 µL (25 µg)	210 °C (hold 1 min) to 350 °C with 6 °C/min (hold 4 min)
8	3 % OV1 on Gaschrom Q (Chrompack) 0.5 m x 2 mm df = 125/150 µm	N_2 40 mL/min	injector 370° C FID 370 °C	calibrant 1 µL (25 µg) sample 0.5 µL (25 µg)	210 °C (hold 1 min) to 350 °C with 6 °C/min (hold 5 min)
9	CP Sil 5 CB (Chrompack) 2.5 m x 0.25 mm df = 0.12 µm	He 60 kPa	cool on-column 110 °C (hold 1 min) to 320 °C with 50 °C/min FID 350 °C	calibrant 1 µL (2 µg) sample 1 µL (2 µg)	100 °C (hold 1 min) to 230 °C with 20 °C/min to 315 °C with 5 °C/min
10	HT Simdist DB (Chrompack) 5 m x 0.53 mm df = 0.17 m	He 60 kPa	cool on-column 110° C (hold 1 min) to 320 °C with 50 °C/min FID 350 °C	calibrant (2 µg) sample (2 µg)	90 °C (hold 1 min) to 230 °C with 20 °C/min to 315 °C with 5 °C/min
11	3 % OV1 on Gaschrom Q (Chrompack) 0.5 m x 2 mm df = 125/150 µm	He 17 mL/min	injector 370 °C FID 370° C	calibrant (25 µg) sample (50 µg)	210 °C (hold 1 min) to 350 °C with 6 °C/min (hold 5 min)

12 ANNEX B (Individual Results of the Certification Exercise)

NOTE: The tables show the results as reported by the laboratories. The results were then subjected to recalculation. For details refer to Section 7.3.

Table B-1: Individual results of laboratory 1 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.0564	0.0558	0.0556	0.0503	0.0498	0.0496	0.0580	0.0572	0.0579	0.0564	0.0558	0.0556
Chol.	0.2628	0.2624	0.2600	0.2543	0.2539	0.2516	0.2486	0.2475	0.2490	0.2628	0.2624	0.2600
C26	0.2694	0.2686	0.2651	0.2591	0.2584	0.2550	0.2756	0.2728	0.2795	0.2694	0.2686	0.2651
C28	0.6991	0.6987	0.7012	0.6901	0.6898	0.6922	0.7148	0.7100	0.7198	0.6991	0.6987	0.7012
C30	1.3362	1.3402	1.3424	1.3455	1.3495	1.3517	1.4235	1.4252	1.4366	1.3362	1.3402	1.3424
C32	2.8471	2.8454	2.8526	2.8705	2.8688	2.8761	3.2919	3.2942	3.3023	2.8471	2.8454	2.8526
C34	6.0622	6.0819	6.0864	6.4908	6.5119	6.5167	6.5540	6.5591	6.5642	6.0622	6.0819	6.0864
C36	10.8144	10.8357	10.8277	10.7466	10.7677	10.7598	11.2173	11.2205	11.1807	10.8144	10.8357	10.8277
C38	12.1688	12.1884	12.1819	12.5075	12.5277	12.5210	12.3781	12.3798	12.3728	12.1688	12.1884	12.1819
C40	9.6357	9.6441	9.6449	9.8994	9.9081	9.9089	9.6680	9.6689	9.6455	9.6357	9.6441	9.6449
C42	6.6065	6.6103	6.5929	7.1060	7.1101	7.0913	6.6101	6.6115	6.6063	6.6065	6.6103	6.5929
C44	6.5145	6.5147	6.5366	6.6713	6.6715	6.6940	6.4326	6.4326	6.4324	6.5145	6.5147	6.5366
C46	7.1723	7.1690	7.1633	7.3059	7.3025	7.2967	6.9556	6.9558	6.9564	7.1723	7.1690	7.1633
C48	8.3876	8.3800	8.3726	8.3178	8.3103	8.3029	8.0571	8.0574	8.0527	8.3876	8.3800	8.3726
C50	10.4792	10.4661	10.4603	10.3408	10.3279	10.3221	10.0834	10.0804	10.077	10.4792	10.4661	10.4603
C52	9.5446	9.5239	9.5285	9.3992	9.3787	9.3833	9.1090	9.1088	9.1166	9.5446	9.5239	9.5285
C54	4.5050	4.4703	4.4890	4.4270	4.3929	4.4113	4.2412	4.2385	4.2710	4.5050	4.4703	4.4890

Table B-2: Individual results of laboratory 2 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.05	0.05	0.05	0.05	0.05	0.05	0.06	0.05	0.05	0.06	0.05	0.05
Chol.	0.27	0.27	0.26	0.26	0.28	0.27	0.26	0.25	0.26	0.26	0.26	0.26
C26	0.32	0.31	0.29	0.32	0.33	0.34	0.34	0.32	0.33	0.34	0.32	0.35
C28	0.73	0.71	0.69	0.74	0.74	0.74	0.73	0.74	0.73	0.75	0.72	0.76
C30	1.36	1.33	1.30	1.34	1.33	1.32	1.41	1.44	1.41	1.45	1.39	1.45
C32	2.89	2.87	2.77	2.84	2.83	2.81	3.24	3.27	3.25	3.33	3.24	3.29
C34	6.29	6.25	6.05	6.24	6.21	6.17	6.71	6.62	6.69	6.72	6.48	6.71
C36	10.98	10.97	10.74	11.00	10.97	10.95	11.33	11.28	11.37	11.33	11.07	11.32
C38	12.53	12.53	12.43	12.54	12.50	12.49	12.67	12.75	12.67	12.62	12.54	12.64
C40	9.97	9.95	10.13	10.00	10.02	9.97	9.99	10.01	10.03	9.93	10.01	9.94
C42	6.97	6.96	7.27	6.99	7.00	6.98	7.00	7.00	7.00	7.03	7.21	6.98
C44	6.68	6.66	6.81	6.64	6.67	6.64	6.57	6.52	6.55	6.53	6.71	6.52
C46	7.35	7.36	7.49	7.34	7.39	7.39	7.16	7.14	7.13	7.15	7.24	7.12
C48	8.81	8.79	8.82	8.78	8.81	8.78	8.53	8.57	8.52	8.44	8.48	8.51
C50	10.83	10.98	10.73	10.89	10.87	10.98	10.48	10.51	10.43	10.51	10.52	10.53
C52	9.57	9.50	9.64	9.58	9.59	9.68	9.25	9.25	9.27	9.21	9.16	9.26
C54	4.40	4.51	4.54	4.45	4.41	4.44	4.27	4.28	4.31	4.33	4.58	4.29

Table B-3: Individual results of laboratory 3 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.05	0.06	0.06	0.05	0.04	0.05	0.05	0.07	0.07	0.05	0.06	0.06
Chol.	0.32	0.32	0.31	0.33	0.33	0.33	0.31	0.31	0.31	0.31	0.31	0.32
C26	0.40	0.40	0.40	0.41	0.41	0.41	0.41	0.44	0.41	0.43	0.42	0.43
C28	0.80	0.81	0.80	0.82	0.82	0.82	0.81	0.82	0.85	0.83	0.84	0.84
C30	1.39	1.33	1.37	1.40	1.39	1.39	1.46	1.47	1.53	1.47	1.50	1.46
C32	2.91	2.85	2.91	2.93	2.93	2.93	3.39	3.40	3.44	3.40	3.44	3.42
C34	6.27	6.22	6.24	6.29	6.29	6.28	6.73	6.71	6.77	6.77	6.78	6.80
C36	10.81	10.83	10.79	10.89	10.88	10.88	11.15	11.17	11.14	11.26	11.30	11.38
C38	12.85	12.90	12.86	12.85	12.86	12.80	13.05	13.05	12.97	13.08	13.09	13.21
C40	10.29	10.30	10.31	10.25	10.26	10.32	10.29	10.33	10.28	10.28	10.29	10.31
C42	7.20	7.21	7.20	7.11	7.08	7.10	7.24	7.22	7.23	7.15	7.14	7.16
C44	6.62	6.64	6.67	6.56	6.55	6.57	6.57	6.54	6.57	6.52	6.47	6.46
C46	7.18	7.25	7.18	7.10	7.18	7.12	7.03	6.99	6.93	6.87	6.89	6.86
C48	8.47	8.46	8.47	8.49	8.46	8.54	8.18	8.21	8.22	8.17	8.19	8.10
C50	10.42	10.59	10.47	10.49	10.52	10.45	9.96	10.03	9.97	9.98	9.93	9.94
C52	9.49	9.36	9.50	9.51	9.54	9.47	9.07	8.99	8.97	9.11	9.07	8.99
C54	4.51	4.47	4.47	4.50	4.44	4.51	4.32	4.26	4.35	4.31	4.28	4.28

Table B-4: Individual results of laboratory 4 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.06	0.06	0.06	0.07	0.06	0.06	0.07	0.07	0.06	0.06	0.07	0.06
Chol.	0.31	0.30	0.30	0.31	0.32	0.31	0.30	0.29	0.30	0.31	0.30	0.30
C26	0.26	0.28	0.27	0.27	0.29	0.27	0.29	0.28	0.28	0.29	0.28	0.29
C28	0.63	0.64	0.63	0.64	0.64	0.64	0.66	0.65	0.65	0.66	0.66	0.67
C30	1.27	1.27	1.28	1.28	1.29	1.28	1.35	1.36	1.37	1.38	1.37	1.36
C32	2.70	2.70	2.72	2.70	2.70	2.70	3.14	3.14	3.15	3.18	3.16	3.16
C34	5.95	5.95	5.98	5.95	5.95	5.94	6.44	6.42	6.43	6.47	6.46	6.43
C36	10.56	10.56	10.60	10.55	10.55	10.52	10.96	10.93	10.92	10.98	10.97	10.93
C38	12.57	12.54	12.57	12.52	12.51	12.50	12.77	12.74	12.73	12.75	12.76	12.74
C40	10.17	10.16	10.17	10.12	10.12	10.12	10.18	10.17	10.15	10.16	10.17	10.16
C42	7.13	7.13	7.14	7.11	7.10	7.10	7.14	7.14	7.14	7.13	7.13	7.16
C44	6.68	6.68	6.68	6.66	6.66	6.66	6.56	6.56	6.56	6.55	6.55	6.57
C46	7.38	7.38	7.37	7.37	7.36	7.38	7.12	7.14	7.15	7.14	7.13	7.16
C48	8.78	8.78	8.74	8.78	8.78	8.80	8.44	8.45	8.45	8.44	8.45	8.47
C50	10.71	10.73	10.66	10.74	10.73	10.75	10.24	10.28	10.28	10.23	10.27	10.25
C52	9.87	9.87	9.83	9.92	9.92	9.95	9.46	9.49	9.50	9.46	9.49	9.45
C54	4.96	4.97	5.00	5.02	5.01	5.02	4.87	4.87	4.88	4.79	4.79	4.86

Table B-5: Individual results of laboratory 5 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.156	0.158	0.153	0.166	0.158	0.157	0.154	0.151	0.151	0.156	0.152	0.155
Chol.	0.273	0.274	0.272	0.268	0.264	0.264	0.261	0.265	0.261	0.252	0.252	0.252
C26	0.369	0.368	0.366	0.361	0.365	0.356	0.388	0.392	0.386	0.374	0.374	0.375
C28	0.753	0.754	0.750	0.746	0.737	0.738	0.770	0.764	0.770	0.755	0.756	0.757
C30	1.408	1.411	1.405	1.400	1.392	1.394	1.493	1.498	1.494	1.478	1.478	1.480
C32	2.791	2.798	2.788	2.778	2.764	2.766	3.230	3.233	3.230	3.195	3.192	3.200
C34	5.821	5.846	5.821	5.824	5.792	5.797	6.288	6.285	6.289	6.240	6.232	6.245
C36	10.021	10.064	10.018	10.098	10.042	10.053	10.400	10.380	10.408	10.391	10.377	10.395
C38	11.968	12.016	11.965	12.019	11.953	11.962	12.187	12.163	12.197	12.137	12.119	12.138
C40	9.860	9.876	9.851	9.908	9.866	9.870	9.903	9.893	9.905	9.895	9.883	9.902
C42	7.086	7.081	7.076	7.011	7.004	7.005	7.116	7.110	7.119	7.036	7.034	7.043
C44	7.026	7.019	7.024	7.032	7.031	7.029	6.930	6.929	6.932	6.934	6.938	6.939
C46	7.854	7.843	7.848	7.849	7.856	7.854	7.628	7.627	7.621	7.636	7.643	7.637
C48	9.209	9.200	9.210	9.091	9.095	9.092	8.891	8.878	8.887	8.785	8.784	8.779
C50	10.729	10.711	10.732	10.781	10.795	10.788	10.293	10.293	10.288	10.370	10.371	10.366
C52	9.653	9.632	9.677	9.794	9.852	9.838	9.259	9.265	9.253	9.461	9.481	9.452
C54	5.022	4.948	5.044	4.873	5.046	5.034	4.808	4.853	4.808	4.905	4.936	4.885

Table B-6: Individual results of laboratory 6 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.07	0.07	0.06	0.07	0.06	0.06	0.07	0.07	0.07	0.08	0.08	0.07
Chol.	0.30	0.30	0.30	0.30	0.30	0.30	0.29	0.29	0.30	0.29	0.29	0.29
C26	0.31	0.32	0.31	0.31	0.31	0.31	0.32	0.32	0.33	0.33	0.32	0.31
C28	0.70	0.70	0.70	0.70	0.70	0.70	0.71	0.71	0.72	0.72	0.71	0.70
C30	1.29	1.29	1.28	1.27	1.27	1.26	1.37	1.38	1.37	1.36	1.37	1.35
C32	2.71	2.72	2.70	2.72	2.69	2.69	3.18	3.18	3.19	3.18	3.20	3.16
C34	6.05	6.07	6.06	6.06	6.03	6.03	6.55	6.54	6.57	6.56	6.59	6.52
C36	10.85	10.88	10.86	10.87	10.85	10.87	11.29	11.29	11.31	11.30	11.32	11.25
C38	12.83	12.86	12.87	12.85	12.87	12.85	13.10	13.11	13.11	13.10	13.12	13.09
C40	10.15	10.15	10.16	10.16	10.17	10.16	10.20	10.19	10.18	10.17	10.17	10.19
C42	6.97	6.96	6.94	6.95	6.95	6.98	6.99	6.98	6.96	6.97	6.97	7.00
C44	6.43	6.44	6.43	6.43	6.44	6.44	6.32	6.32	6.30	6.32	6.31	6.34
C46	7.10	7.11	7.10	7.10	7.11	7.09	6.86	6.86	6.84	6.86	6.86	6.89
C48	8.67	8.67	8.67	8.67	8.67	8.68	8.34	8.35	8.34	8.35	8.35	8.37
C50	10.79	10.76	10.80	10.78	10.80	10.79	10.31	10.32	10.31	10.32	10.30	10.35
C52	10.04	10.00	10.03	10.03	10.04	10.05	9.57	9.59	9.57	9.56	9.56	9.60
C54	4.73	4.71	4.72	4.72	4.73	4.73	4.52	4.51	4.50	4.51	4.49	4.52

Table B-7: Individual results of laboratory 7 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.07	0.07	0.07	0.07	0.07	0.08	0.09	0.09	0.08	0.08	0.08	0.08
Chol.	0.30	0.29	0.29	0.29	0.29	0.28	0.27	0.29	0.28	0.27	0.27	0.27
C26	0.38	0.40	0.39	0.39	0.39	0.40	0.42	0.44	0.42	0.41	0.41	0.41
C28	0.83	0.85	0.83	0.85	0.85	0.83	0.84	0.89	0.87	0.85	0.85	0.86
C30	1.47	1.47	1.47	1.50	1.51	1.48	1.55	1.63	1.58	1.59	1.58	1.61
C32	2.90	2.89	2.89	2.95	2.97	2.93	3.34	3.42	3.40	3.40	3.37	3.41
C34	5.98	5.96	5.97	6.02	6.05	6.01	6.40	6.48	6.49	6.50	6.44	6.46
C36	10.15	10.12	10.15	10.23	10.22	10.12	10.51	10.55	10.53	10.59	10.51	10.54
C38	11.99	11.94	11.96	11.94	12.07	11.99	12.15	12.18	12.21	12.20	12.19	12.16
C40	9.83	9.82	9.84	9.82	9.80	9.82	9.87	9.89	9.92	9.85	9.82	9.88
C42	7.08	7.11	7.11	7.12	7.12	7.14	7.11	7.13	7.12	7.18	7.19	7.14
C44	6.79	6.78	6.78	6.76	6.78	6.75	6.69	6.70	6.71	6.66	6.69	6.67
C46	7.48	7.53	7.49	7.43	7.47	7.41	7.26	7.28	7.28	7.18	7.20	7.26
C48	8.82	8.85	8.83	8.74	8.70	8.76	8.54	8.45	8.49	8.45	8.46	8.45
C50	10.91	10.96	11.11	10.77	10.78	10.83	10.48	10.45	10.43	10.34	10.39	10.37
C52	10.16	10.11	10.04	10.18	10.14	10.21	9.81	9.59	9.60	9.69	9.73	9.62
C54	4.87	4.85	4.79	4.95	4.80	4.95	4.69	4.54	4.60	4.73	4.82	4.80

Table B-8: Individual results of laboratory 8 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.06	0.06	0.06	0.06	0.05	0.06	0.06	0 .06	0.06	0.05	0.06	0.05
Chol.	0.31	0.30	0.30	0.31	0.31	0.30	0.29	0.29	0.32	0.29	0.29	0.29
C26	0.26	0.26	0.26	0.26	0.26	0.26	0.27	0.28	0.30	0.27	0.27	0.27
C28	0.67	0.66	0.66	0.67	0.67	0.66	0.67	0.68	0.70	0.67	0.68	0.68
C30	1.28	1.27	1.27	1.28	1.28	1.28	1.35	1.36	1.40	1.35	1.37	1.37
C32	2.74	2.72	2.72	2.73	2.73	2.72	3.18	3.20	3.23	3.18	3.21	3.21
C34	6.00	5.99	5.95	5.98	5.98	5.97	6.43	6.45	6.46	6.43	6.49	6.50
C36	10.70	10.67	10.69	10.65	10.66	10.62	11.07	11.10	11.05	11.07	11.16	11.17
C38	12.74	12.72	12.63	12.68	12.71	12.66	12.93	12.96	12.87	12.94	13.02	13.03
C40	10.15	10.14	10.10	10.23	10.24	10.22	10.17	10.19	10.13	10.26	10.32	10.33
C42	7.09	7.08	7.07	7.09	7.08	7.09	7.13	7.13	7.11	7.12	7.13	7.15
C44	6.65	6.65	6.66	6.67	6.65	6.66	6.55	6.55	6.55	6.56	6.54	6.55
C46	7.36	7.37	7.39	7.39	7.38	7.38	7.14	7.14	7.13	7.15	7.12	7.12
C48	8.79	8.81	8.82	8.82	8.82	8.8	8.47	8.48	8.44	8.50	8.44	8.44
C50	10.74	10.78	10.78	10.77	10.79	10.75	10.28	10.3	10.23	10.34	10.25	10.24
C52	9.83	9.86	9.87	9.85	9.86	9.85	9.40	9.41	9.36	9.45	9.37	9.34
C54	4.66	4.67	4.76	4.68	4.66	4.71	4.63	4.47	4.64	4.48	4.42	4.41

Table B-9: Individual results of laboratory 9 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05	0.05
Chol.	0.30	0.30	0.28	0.31	0.31	0.31	0.28	0.28	0.26	0.29	0.29	0.30
C26	0.27	0.26	0.25	0.28	0.27	0.28	0.27	0.27	0.26	0.28	0.28	0.29
C28	0.69	0.67	0.65	0.67	0.66	0.67	0.67	0.68	0.65	0.66	0.65	0.68
C30	1.28	1.25	1.22	1.32	1.30	1.33	1.33	1.33	1.29	1.36	1.36	1.39
C32	2.70	2.65	2.60	2.77	2.74	2.78	3.09	3.10	3.04	3.16	3.14	3.20
C34	5.95	5.87	5.80	6.04	6.00	6.04	6.41	6.40	6.35	6.47	6.46	6.51
C36	10.55	10.47	10.40	10.60	10.56	10.58	10.93	10.89	10.88	10.98	10.93	10.99
C38	12.58	12.52	12.51	12.54	12.51	12.50	12.75	12.73	12.75	12.80	12.76	12.77
C40	10.17	10.15	10.17	10.11	10.11	10.08	10.20	10.15	10.19	10.21	10.18	10.17
C42	7.11	7.12	7.14	7.03	7.03	7.01	7.16	7.14	7.17	7.10	7.09	7.08
C44	6.65	6.67	6.70	6.62	6.62	6.61	6.58	6.56	6.59	6.56	6.56	6.54
C46	7.33	7.36	7.40	7.31	7.32	7.31	7.15	7.14	7.17	7.13	7.13	7.11
C48	8.79	8.84	8.87	8.75	8.78	8.76	8.50	8.50	8.53	8.47	8.49	8.45
C50	10.74	10.81	10.86	10.72	10.75	10.73	10.32	10.34	10.37	10.28	10.33	10.27
C52	9.88	9.98	10.03	9.89	9.94	9.92	9.51	9.57	9.56	9.44	9.50	9.44
C54	4.94	5.02	5.08	5.01	5.04	5.05	4.80	4.87	4.89	4.76	4.80	4.76

Table B-10: Individual results of laboratory 10 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24 + chol.	0.34	0.34	0.34	0.34	0.34	0.34	0.33	0.33	0.33	0.33	0.33	0.33
C26	0.34	0.33	0.33	0.34	0.33	0.34	0.34	0.35	0.36	0.35	0.35	0.35
C28	0.75	0.75	0.75	0.76	0.75	0.75	0.76	0.76	0.77	0.76	0.76	0.77
C30	1.42	1.41	1.41	1.42	1.40	1.41	1.50	1.51	1.52	1.51	1.50	1.51
C32	2.94	2.94	2.93	2.95	2.92	2.93	3.43	3.42	3.44	3.41	3.40	3.41
C34	6.30	6.29	6.28	6.26	6.26	6.27	6.80	6.77	6.78	6.76	6.74	6.74
C36	10.88	10.89	10.82	10.84	10.81	10.84	11.31	11.23	11.21	11.18	11.19	11.22
C38	12.85	12.82	12.82	12.82	12.79	12.81	13.04	13.02	13.02	13.01	12.97	12.98
C40	10.24	10.24	10.22	10.27	10.26	10.21	10.28	10.24	10.21	10.24	10.23	10.25
C42	7.06	7.10	7.07	7.11	7.13	7.07	7.11	7.11	7.13	7.16	7.15	7.12
C44	6.56	6.58	6.57	6.56	6.58	6.56	6.43	6.47	6.47	6.48	6.46	6.47
C46	7.17	7.17	7.17	7.14	7.16	7.19	6.88	6.94	6.94	6.96	6.96	6.94
C48	8.58	8.60	8.64	8.59	8.60	8.61	8.22	8.28	8.28	8.29	8.27	8.28
C50	10.57	10.53	10.58	10.53	10.53	10.54	10.05	10.13	10.08	10.12	10.11	10.09
C52	9.64	9.60	9.62	9.55	9.61	9.59	9.19	9.18	9.22	9.18	9.20	9.17
C54	4.36	4.44	4.46	4.51	4.52	4.53	4.30	4.26	4.24	4.25	4.36	4.37

Table B-11: Individual results of laboratory 11 in the certification exercise.

TG	A-1	A-2	A-3	A-4	A-5	A-6	B-1	B-2	B-3	B-4	B-5	B-6
C24	0.05	0.05	0.05	0.05	0.04	0.04	0.06	0.06	0.05	0.05	0.05	0.05
Chol.	0.36	0.36	0.36	0.35	0.35	0.36	0.35	0.35	0.35	0.35	0.34	0.35
C26	0.28	0.28	0.28	0.28	0.28	0.28	0.29	0.29	0.29	0.29	0.29	0.29
C28	0.66	0.65	0.65	0.64	0.65	0.65	0.67	0.66	0.66	0.65	0.66	0.66
C30	1.27	1.28	1.27	1.25	1.26	1.27	1.37	1.36	1.35	1.35	1.35	1.36
C32	2.68	2.68	2.69	2.69	2.68	2.70	3.17	3.16	3.15	3.16	3.16	3.16
C34	5.87	5.93	5.91	5.96	5.93	5.96	6.41	6.41	6.42	6.42	6.39	6.40
C36	10.83	10.77	10.87	10.89	10.85	10.89	11.23	11.23	11.20	11.27	11.28	11.24
C38	12.87	12.86	12.83	12.96	12.92	12.95	13.13	13.13	13.08	13.16	13.13	13.10
C40	10.22	10.19	10.21	10.27	10.27	10.27	10.26	10.25	10.25	10.24	10.23	10.27
C42	6.90	6.91	6.90	6.92	6.91	6.89	6.92	6.94	6.95	6.92	6.93	6.96
C44	6.39	6.44	6.38	6.41	6.40	6.39	6.28	6.29	6.33	6.29	6.27	6.34
C46	7.01	7.01	7.05	7.04	7.05	6.98	6.75	6.76	6.79	6.78	6.78	6.81
C48	8.47	8.51	8.48	8.46	8.41	8.42	8.15	8.16	8.19	8.16	8.15	8.20
C50	10.75	10.72	10.72	10.69	10.77	10.68	10.30	10.22	10.32	10.30	10.30	10.24
C52	10.38	10.31	10.32	10.20	10.23	10.30	9.84	9.90	9.84	9.83	9.89	9.81
C54	5.03	5.05	5.02	4.93	4.99	4.98	4.82	4.81	4.80	4.76	4.79	4.76

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Abstract

This report describes the preparation of a set of two butter fat reference materials (pure and adulterated butter fat) and the measurement exercises that led to the method-specific certification of the content (relative mass fraction) of the triglycerides C24 - C54 (even numbers) and cholesterol. The content of the triglycerides and of cholesterol has been normalised to 100 g. The relative mass fractions are presented in Table 1. The results of the certification exercise, which involved 11 European laboratories, are presented and discussed. Uncertainties were calculated in compliance with the Guide to the Expression of Uncertainty in Measurement (GUM) including uncertainties due to possible inhomogeneity and possible instability as well as uncertainty contributions from the calibrant (impurities and weighing).

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